

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. : 10/600,266
Applicant(s) : Fumitoshi Asai et al
Filed : June 20, 2003
For : Medicinal Compositions Containing Aspirin
Group Art Unit : 1629
Examiner : Leslie A Royds Draper
Docket No. : 17620.105003
Confirmation No.: 7488

DECLARATION UNDER 37 CFR 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SIR:

The below named declarants hereby declare the following:

1. We are each a co-inventor of the invention described and claimed in the above-identified application.

2. Attached hereto are copies of notebook records documenting experiments done by us (the inventors) or under our supervision and control, showing reduction to practice of the claimed invention. English-language translations of these documents are also attached. The dates on the attached copies have been redacted. The dates deleted from the attachments are prior to November 3, 1998.

3. The code "CS-747", "CS747", and "747", which appear throughout the attached copies of the notebook page are our internal code for the compound identified as "Compound A" in the specification of the above-identified application.

4. The acts described in the attached documents report work done in Japan which occurred prior to November 3, 1998.

5. This Declaration under 37 CFR 1.131 is essentially the same declaration signed by Dr. Asai on October 11, 2007 and filed in U.S. Application Serial No. 10/600,266. It has now recently come to our attention that the copies of the Japanese language notebook pages that were affixed to the final version of the previous declarations which were filed in the United States Patent and Trademark Office in October 2007 were missing the even numbered notebook pages. However, the English language translation of the Japanese language notebook pages that was also affixed to the declarations and that was filed with the original declarations included translations of the missing Japanese language notebook pages. The copies of the Japanese language notebook pages attached to this present declaration contains both odd and even numbered pages and is a complete set of the notebook pages translated and attached to the declaration as well. The English language translation is the same as that previously submitted in October 2007. The error that resulted in the missing notebook pages occurred without deceptive intent.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11/24/2011



Fumitoshi Asai, Ph.D.

Date: 11/24/2011



Atsuhiro Sugidachi, Ph.D

Date: 11/24/2011

T. Ogawa
Taketoshi Ogawa, Ph.D

Date: 11/21/2011

Teruhiko Inoue
Teruhiko Inoue

Attachment: Notebook records and
English translation thereof

145

CS 747
+
Aspirin

備用 Exp. 行なう。

Dose CS 747 0.3 mg/kg (4hr) ～ 約 40 mg.
1 mg/kg (4hr) ～ 約 20

出血時間、凝集とも行なう。dose. 12

Aspirin ⑩ + CS 747 ⑩.6 2hr

まち Aspirin ⑩ + CS 747 ⑩.6 を中心で行なう。

まち 12 ⑩.3 ～ ① ～ ① は 5 分 間をもって ⑩.3 と行なう。

ラット動静脈シャント血栓モデル (CS-747とaspirinの併用効果検討)

[目的]

ラット動静脈シャント血栓モデルを用いてCS-747とaspirinの併用効果を検討する。

[実験期間]

[]に入荷のラット (36匹) を用いる。

[動物]

雄性SD系ラット (日本SLC)。7週齢で購入し、約1週間予備飼育の後実験に用いる。実験は1群6匹として行う。

[薬物]

CS-747 (宇部興産合成、Lot No. 16) およびaspirin (Sigma、A-5376、Lot No. 46H1053、[]入荷) を用いる。薬物は5%アラビアゴム (Sigma、Lot No. 73H0705、[]開封) 溶液に溶解あるいは懸濁させ、1 ml/kgの割合で動静脈シャント開通の2時間前に経口投与する。投与群は、(A) vehicle、(B) aspirin 10 mg/kg、(C) CS-747 0.3 mg/kg、(D) CS-747 0.6 mg/kg、(E) aspirin 10 mg/kg + CS-747 0.3 mg/kg、(F) aspirin 10 mg/kg + CS-747 0.6 mg/kgとする。

[方法]

(1) 実験はUmetsuらの方法 (Thromb. Haemost. 39, 74-83, 1978) を部分的に改変して行なう。

(2) 動静脈シャントに用いるチューブを以下のように作製する。長さ12 cmのメディカルシリコンチューブ (内径1.5 mm、外径2.5 mm、カネカメディックス) の両端に7 cmのシリコン (L-25、富士システムズ^社処理したポリエチレンチューブ (内径0.5 mm、外径1.0 mm、夏目製作所) を取り付ける。ジョイントとして0.7 cmに切断したメディカルシリコンチューブ (内径1.0 mm、外径1.5 mm、カネカメディックス) を用い、接続部は血液が漏れないように外科用接着剤 (ア

147

ロンアルファA、三共)で接着する。また12 cmのチューブ内に10 cmの綿糸(3-0、日腸工業)を設置する。

(3) 動静脈シャント開始2時間前に、vehicle (5%アラビアゴム溶液) または薬物を1 ml/kgの割合で経口投与する。1群6匹の実験を行う。

(4) あらかじめ作成しておいた上記チューブに30 unit/kgとなるように、生理食塩液(大塚)で希釈したヘパリン溶液(日本薬局方ヘパリンナトリウム注射液、扶桑薬品工業、Lot No. 97H28A、[REDACTED] 入荷)を満たす。

(5) 生理食塩液で40 mg/mlに希釈したペントバルビタール溶液(ネンプタール®、Abbott、Lot No. 20-975-Z7)を1 ml/kg腹腔内投与(40 mg/kg)してラットを麻酔させる。仰臥位に固定させた後、頸静脈を露出させ、シャント用チューブの片側(糸の付いていない方)をカニュレーションする。続いて、クレンメで血流を遮断しておいた頸動脈に、チューブの反対端をカニュレーションし、動静脈シャントを形成する。

(6) クレンメをはずしてシャントに30分間血液を循環させた後、綿糸に付着した血栓の湿重量を測定する。測定重量から糸の重量(6.5 mg)を差し引き血栓重量を求める。

ファイル AV-shunt(3) 1=保存
(E00515データ)

A: Sigitario

ラット AV shunt 白椎玉子
(アスピリンとCS747併用)

70ロトコール P146, 147

試験等 P110, P138

ケージ番号: 65C3-01-04

性別、系統: ♂ SD

周年令: 7週

体重:

3 楽者名: 日本SLC

入荷日: [REDACTED]

実験者: 杉立 収寛

飼育期間: [REDACTED]

受付番号: 034163

匹数: 36

ラット

SD ♂ (日本SLC)

7wks 入荷

受付 No. 034163

杉立

5% Arabic gum soln.

$$50 \text{ mg/ml} = 1011.7 \text{ mg} / 20.234 \text{ ml dH}_2\text{O}$$

57.7 mg アスピリン

16.2 mg CS747

1011.7 mg アスピリン

Aspirin

$$10 \text{ mg/ml} = 57.7 \text{ mg} / 5.77 \text{ ml} \cdot 5\% \text{ アスピリン soln.}$$

CS747

$$16.2 \text{ mg} / 5.4 \text{ ml} = 3 \text{ mg/ml}$$

$$3 \text{ mg/ml soln. } 1 \text{ ml} + 5\% \text{ アスピリン soln. } 2 \text{ ml} = 1 \text{ mg/ml}$$

$$1 \text{ mg/ml soln. } 1 \text{ ml} + 5\% \text{ アスピリン soln. } 2.33 \text{ ml} = 0.3 \text{ mg/ml}$$

$$1 \text{ mg/ml soln. } 1.5 \text{ ml} + 5\% \text{ アスピリン soln. } 1 \text{ ml} = 0.6 \text{ mg/ml}$$

たゞこので 75% 作成 (二重作成)

$$5\% \text{ アスピリン } 1084.7 \text{ mg} / 21.69 \text{ ml dH}_2\text{O} = 50 \text{ mg/ml}$$

$$CS747 10.7 \text{ mg} / 10.7 \text{ ml アスピリン soln.} = 1 \text{ mg/ml}$$

$$1 \text{ mg/ml soln. } 1.5 \text{ ml} + 5\% \text{ アスピリン soln. } 3.5 \text{ ml} = 0.3 \text{ mg/ml}$$

$$1 \text{ mg/ml soln. } 3 \text{ ml} + 5\% \text{ アスピリン soln. } 2 \text{ ml} = 0.6 \text{ mg/ml}$$

149

| B.W. (g) | | Treatment | 測定値 (ms) | Thrombus (mg) |
|----------|-----|----------------------------|----------|---------------|
| #1 | 253 | Vehicle | 61.3 | 54.8 |
| 2 | 252 | Aspirin (10) | 51.3 | 44.8 |
| 3 | 262 | CS747 (0.3) | 58.3 | 51.8 |
| 4 | 267 | CS747 (0.6) | 43.1 | 36.6 |
| 5 | 256 | Aspirin (10) + CS747 (0.3) | 39.6 | 33.1 |
| 6 | 271 | Aspirin (10) + CS747 (0.6) | 23.2 | 16.7 |
| 7 | 250 | V | 63.2 | 56.7 |
| 8 | 246 | A (10) | 58.4 | 51.9 |
| 9 | 258 | 747 (0.3) | 51.8 | 45.3 |
| 10 | 269 | 747 (0.6) | 53.1 | 46.6 |
| 11 | 268 | A (10) + 747 (0.3) | 30.5 | 24.0 |
| 12 | 244 | A (10) + 747 (0.6) | 41.3 | 34.8 |
| 13 | 247 | V | 56.1 | 49.6 |
| 14 | 262 | A (10) | 48.6 | 42.1 |
| 15 | 256 | 747 (0.3) | 52.2 | 45.7 |
| 16 | 267 | 747 (0.6) | 46.3 | 39.8 |
| 17 | 268 | A (10) + 747 (0.3) | 42.2 | 35.7 |
| 18 | 242 | A (10) + 747 (0.6) | 21.6 | 15.1 |

V = Vehicle

A = Aspirin

747 = CS747

#1

252.9g
251.7g
262.3g
267.3g
256.4g
271.1g

#5

#7

250.0g
245.8g
257.8g
269.0g
268.3g
244.4g

#6

#13

247.8g
262.3g
256.2g
267.3g
267.6g
242.1g

~10°)

1000 u/ml soln. 1ml + saline 9ml = 100 unit/ml

100 u/ml soln. 3ml + saline 7ml = 30 unit/ml

150

#1 0.0613 g

#7 0.0632 g

#13 0.0561 g

#2 0.0513 g

#8 0.0594 g

#14 48.6 mg

#3 0.0583 g

#9 0.0518 g

#15 52.2 mg

#4 0.0431 g

#10 0.0531 g

#16 0.0463 g

#5 0.0396 g

#11 0.0305 g

#17 0.0422 g

#6 0.0232 g

#12 41.3 mg

#18 0.0216 g

unit/g

g

A. Sugidarki

151

ラット AV shunt 腹腔エリザ
(アスピリル + CS747)

CS747

プロトコル p146, 147 記録 p110, 118

ケージ番号: 65C3-01-01

性別、系統: ♂ SD

周年令: 7週

体重:

6

36+2

匹数: 36

繁殖者名: 日本SLC

入荷日: [REDACTED]

実験者: 杉立 収寛

飼育期間: [REDACTED]

受付番号: 034163

B.W.

#1 271.0g
277.6g
272.8g
275.0g
245.5g
#6 251.6g

#10 248.2g
#8 263.9g
#9 253.3g
#7 281.1g
#11 263.4g
#12 263.4g

#10 271.3g
265.3g
266.0g
255.5g
245.6g
#12 272.4g

72.0 mg
11.3 mg

1224.1 mg

5%アラビアゴム = 50 mg/ml

= 1224.1 / 24.48 ml dH₂O

Aspirin

10 mg/ml = 72.0 / 7.2 ml 5%アラビアゴム so/h.

ラット

A10

SD ♂ (7wks入荷)

日本SLC

B.W.

[REDACTED]入荷

#1 271

2 278

3 273

4 275

5 246

↓

6 252

CO₂ gas = 安寧死

7 281

土せん

8 264

9 253

10 248

11 263

12 263

13 271

14 265

15 266

16 256

17 246

18 272

$$\begin{array}{l}
 \text{CS747} \quad 11.3 \text{ mg} / 11.3 \text{ ml} \quad 5\% \text{ PEG3350 soln.} = 1 \text{ mg/ml} \\
 \left. \begin{array}{l}
 1 \text{ mg/ml soln.} \quad 1.5 \text{ ml} + 5\% \text{ PEG3350 soln.} 1 \text{ ml} = 0.6 \text{ mg/ml} \\
 1 \text{ mg/ml soln.} \quad 1 \text{ ml} + 5\% \text{ PEG3350 soln.} 2.33 \text{ ml} = 0.3 \text{ mg/ml}
 \end{array} \right.
 \end{array}$$

$$\begin{array}{l}
 100 \text{ unit/ml soln. (original)} \quad 1 \text{ ml} + \text{water} 9 \text{ ml} = 100 \text{ unit/ml} \\
 100 \text{ unit/ml soln.} \quad 3 \text{ ml} + \text{water} 7 \text{ ml} = 30 \text{ unit/ml}
 \end{array}$$

荷)

| | B.W. (g) | Treatment | 潜伏期 (ms) | Thrombus (%) |
|------|----------|-----------|----------|--------------|
| 63 | 1 271 | V | 55.6 | 49.1 |
| | 2 278 | A⑩ | 52.9 | 46.4 |
| | 3 273 | 747⑩ | 43.9 | 37.4 |
| | 4 275 | 747⑩ | 41.0 | 34.5 |
| | 5 246 | A⑩ + 747⑩ | 24.6 | 18.1 |
| | 6 252 | A⑩ + 747⑩ | 19.7 | 13.2 |
| 实验动物 | 7 281 | V | 59.0 | 52.5 |
| | 8 264 | A⑩ | 63.2 | 56.7 |
| | 9 253 | 747⑩ | 47.1 | 40.6 |
| | 10 248 | 747⑩ | 38.5 | 32.0 |
| | 11 263 | A⑩ + 747⑩ | 36.7 | 30.2 |
| | 12 263 | A⑩ + 747⑩ | 35.6 | 29.1 |
| | 13 271 | V | 57.8 | 51.3 |
| | 14 265 | A⑩ | 44.3 | 37.8 |
| | 15 266 | 747⑩ | 46.4 | 39.9 |
| | 16 256 | 747⑩ | 42.2 | 35.7 |
| | 17 246 | A⑩ + 747⑩ | 48.3 | 41.8 |
| | 18 272 | A⑩ + 747⑩ | 36.6 | 30.1 |

V = Vehicle

A = Aspirin

747 = CS-747

153

#1 0.0556 g

#7 0.0590 g

#13 0.0578 g

#2 0.0529 g

#8 0.0632 g

#14 44.3 mg

#3 0.0439 g

#9 0.0471 g

#15 0.0464 g

#4 0.0410 g

#10 0.0385 g

#16 42.2 mg

#5 0.0246 g

#11 0.0367 g

#17 48.3 mg

Aspirin +

Vi

#6 0.0197 g

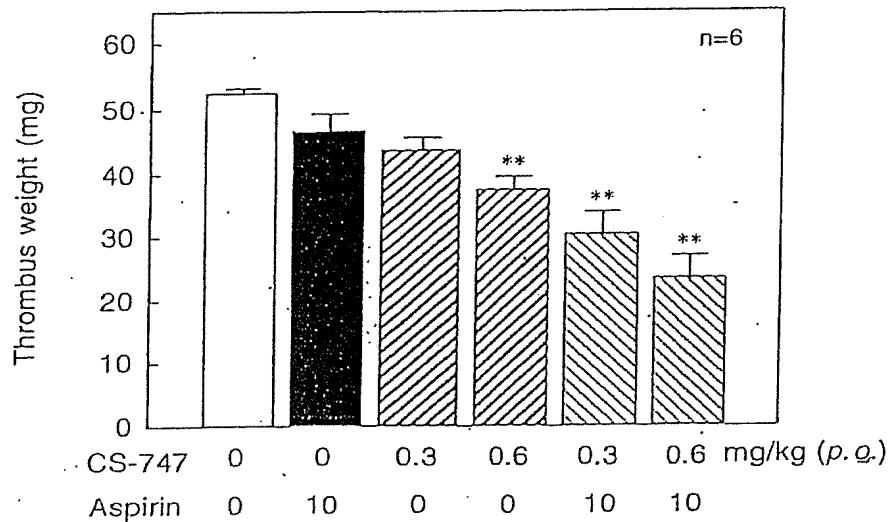
#12 0.0356 g

#18 36.6 mg



A. Sugardahl

Arterio-venous shunt thrombosis model in rats



Aspirin + CS747 test

| | |
|----------------------------|----------------|
| Vehicle | 52.3 ± 1.2 |
| Aspirin (10) | 46.6 ± 2.8 |
| CS747 (0.3) | 43.5 ± 2.1 |
| CS747 (0.6) | 37.5 ± 2.1 |
| Aspirin (10) + CS747 (0.3) | 30.5 ± 3.5 |
| Aspirin (10) + CS747 (0.6) | 23.2 ± 3.8 |

A. Suganthi

CS 747

+

Combination Experiment

Aspirin

| Dose | CS 747 | 0.3 mg/kg (4hr) | about 40 mg |
|------|--------|-----------------|-------------|
| | | 1 mg/kg (4hr) | about 20 |

Dose in which both bleeding time and aggregation were measured:

Aspirin 10 + CS 747 0.6 2hr

First, experiment with a central focus on Aspirin 10 + CS 747 0.6

Another group 0.3 or 1

Conduct 0.3 because 1 seems to work too much.

Arterio-venous Shunt Thrombosis Model in Rats
(Examination of effect by combination of CS-747 with aspirin)

[Object]

Examine the effect by combination of CS-747 with aspirin using Arterio-venous shunt thrombosis model in rats.

[Experimental Term]

Thirty six rats received on [REDACTED] are used.

[Animals]

Seven-week-old male SD rats (Japan SLC) are purchased and used for the experiment after preliminary breeding for about a week. The experiment is conducted as 6 rats per group.

[Test agents]

CS-747 (synthesized by Ube Industrials Ltd., Lot No. 16) and aspirin (Sigma, A-5376, Lot No. 46H1053, received on [REDACTED]) are used. The test agents are dissolved or suspended in a 5% Arabic gum (Sigma, Lot No. 73H0705, opened on [REDACTED]) solution and administered orally in volume of 1 ml/kg two hours before starting arterio-venous shunt. Administered group are (A) vehicle, (B) aspirin 10 mg/kg, (C) CS-747 0.3 mg/kg, (D) CS-747 0.6 mg/kg, (E) aspirin 10 mg/kg + CS-747 0.3 mg/kg, and (F) aspirin 10 mg/kg + CS-747 0.6 mg/kg.

[Methods]

(1) For the experiment, the method by Umetsu et al. (Thromb. Haemost. 39, 74-83, 1978) is partly modified.

(2) The shunt tube for arterio-venous shunt is prepared as follows: both sides of a medical silicon tube of 12 cm length (inner diameter: 1.5 mm, outer diameter: 2.5 mm, KANEKA Medix Co., Ltd) are connected each to a polyethylene tube of 7 cm length (inner diameter: 0.5 mm, outer diameter: 1.0 mm, Natsume Seisakusho Co., Ltd.)

covered with silicon via a medical silicon tube of 0.7 cm length (inner diameter: 1.0 mm, outer diameter: 1.5 mm, KANEKA Medix Co., Ltd.) as connector. At the connection,

Page 147

[REDACTED]

surgical adhesive (Aronalpha A, Sankyo) is used for preventing blood leak. In addition, a silk thread (size 3-0, Niccho Kogyo) of 10 cm length is placed in the tube of 12 cm length.

(3) Vehicle (5% Arabic gum solution) or test agents are administered orally in a volume of 1 ml/kg 2 hours before starting arterio-venous shunt. 6 rats per group are used.

(4) The above tube prepared in advance is filled with heparin solution (Japanese Pharmacopoeia Heparin Sodium Injection, Fuso Pharmaceutical Industries, Ltd., Lot No. 97H28A, received on [REDACTED]) diluted with normal saline (Otsuka) resulting in 30 unit/kg.

(5) The rat is anesthetized with an intraperitoneal injection of 1 ml/kg (40 mg/kg) of pentobarbital solution (Nembutal R, Abbott, Lot No. 20-975-Z7) diluted with normal saline resulting in 40 mg/ml. After it is fixed to turning up, the jugular vein is exposed and one side of the shunt tube (in which the silk thread is not adhered) is cannulated. Subsequently, to the carotid artery where bloodstream is shut using clamp, the other side of the tube is cannulated to make the arterio-venous shunt.

(6) After removing the clamp and allowing blood to circulate for 30 minutes, the thrombus adsorbed on the silk thread is weighed. The thrombus weight was calculated by subtracting of the weight of the thread (6.5 mg) from the measured weight.

Files were stored at AV-shunt (3) (F00515 data)

[REDACTED] A. Sugidachi

AV shunt Thrombosis Model in Rats
(Combination of CS 747 with aspirin)

Protocol: P. 146, 147

Reagent and so on: p. 110, p. 138

Cage number: 6raC3-01-04

Rats

SD male (Japan SLC)

Sex, system: male SD

[REDACTED] wks, Receipt

Year-round old: 7 weeks

Receipt number: 034163

Body weight:

Sugidachi

Manufacture name: Japan SLC

Number of rats: 36

Receive date: [REDACTED]

Experimenter: Atsuhiko Sugidachi

Housing term: [REDACTED] to [REDACTED]

Receipt number: 034163

5% Arabic gum soln.

57.7 mg aspirin

50 mg/ml = 1011.7 mg / 20.234 ml dH₂O

16.2 mg CS 747

Aspirin

10 mg/ml = 57.7 mg / 5.77 ml 5% Arabic gum soln.

1011.7 mg Arabic gum

CS 747

16.2 mg / 5.4 ml = 3 mg/ml

3 mg/ml soln. 1 ml + 5% Arabic gum soln. 2 ml = 1 mg/ml

1 mg/ml soln. 1 ml + 5% Arabic gum soln. 2.33 ml = 0.3 mg/ml

1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 1 ml = 0.6 mg/ml

10.7 mg CS 747

Further prepared because of insufficient (spilled)

5% Arabic gum 1084.7 mg / 21.69 ml dH₂O = 50 mg/ml

1084.7 mg Arabic gum

CS 747 10.7 mg / 10.7 ml Arabic gum soln. = 1 mg/ml

1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 3.5 ml = 0.3 mg/ml

1 mg/ml soln. 3 ml + 5% Arabic gum soln. 2 ml = 0.6 mg/ml

| | B. W. (g) | Treatment | Measured value (mg) | Thrombus (mg) |
|----|-----------|-------------------------|---------------------|---------------|
| #1 | 253 | Vehicle | 61.3 | 54.8 |
| 2 | 252 | Aspirin 10 | 51.3 | 44.8 |
| 3 | 262 | CS 747 0.3 | 58.3 | 51.8 |
| 4 | 267 | CS 747 0.6 | 43.1 | 36.6 |
| 5 | 256 | Aspirin 10 + CS 747 0.3 | 39.6 | 33.1 |
| 6 | 271 | Aspirin 10 + CS 747 0.6 | 23.2 | 16.7 |
| 7 | 250 | V | 63.2 | 56.7 |
| 8 | 246 | A 10 | 58.4 | 51.9 |
| 9 | 258 | 747 0.3 | 51.8 | 45.3 |
| 10 | 269 | 747 0.6 | 53.1 | 46.6 |
| 11 | 268 | A 10 + 747 0.3 | 30.5 | 24.0 |
| 12 | 244 | A 10 + 747 0.6 | 41.3 | 34.8 |
| 13 | 247 | V | 56.1 | 49.6 |
| 14 | 262 | A 10 | 48.6 | 42.1 |
| 15 | 256 | 747 0.3 | 52.2 | 45.7 |
| 16 | 267 | 747 0.6 | 46.3 | 39.8 |
| 17 | 268 | A 10 + 747 0.3 | 42.2 | 35.7 |
| 18 | 242 | A 10 + 747 0.6 | 21.6 | 15.1 |

V = Vehicle

A = Aspirin

#1 252.9 g 747 = CS 747
 251.7 g
 262.3 g
 267.3 g
 256.4 g
 271.1 g heparin
 1000 u/ml soln. 1 ml + saline 9 ml = 100 unit/ml

#7 250.0 g 100 u/ml soln. 3 ml + saline 7 ml = 30 unit/ml
 245.8 g
 257.8 g
 269.0 g
 268.3 g
 244.4 g

#13 247.0 g
 262.3 g
 256.2 g
 267.3 g
 267.6 g
 242.1 g

[REDACTED]

| | | | | | |
|----|----------|----|----------|-----|----------|
| #1 | 0.0613 g | #7 | 0.0632 g | #13 | 0.0561 g |
|----|----------|----|----------|-----|----------|

| | | | | | |
|----|----------|----|----------|-----|---------|
| #2 | 0.0513 g | #8 | 0.0584 g | #14 | 48.6 mg |
|----|----------|----|----------|-----|---------|

| | | | | | |
|----|----------|----|----------|-----|---------|
| #3 | 0.0583 g | #9 | 0.0518 g | #15 | 52.2 mg |
|----|----------|----|----------|-----|---------|

| | | | | | |
|----|----------|-----|----------|-----|----------|
| #4 | 0.0431 g | #10 | 0.0531 g | #16 | 0.0463 g |
|----|----------|-----|----------|-----|----------|

| | | | | | |
|----|----------|-----|----------|-----|----------|
| #5 | 0.0396 g | #11 | 0.0305 g | #17 | 0.0422 g |
|----|----------|-----|----------|-----|----------|

| | | | | | |
|----|----------|-----|---------|-----|----------|
| #6 | 0.0232 g | #12 | 41.3 mg | #18 | 0.0216 g |
|----|----------|-----|---------|-----|----------|

[REDACTED] A. Sugidachi

AV Shunt Thrombosis Model in Rats
(Aspirin + CS 747)

Protocol: p. 146, 147

Reagents and so on: p. 110, 138

| | | |
|-------------------|--------------------|--------------------------|
| Cage number: | 6raC3-01-01 | Rat |
| | | 6 |
| Sex, system: | male SD | SD male (7 wks, Receipt) |
| Year-round old: | 7 weeks | Japan SLC |
| Body weight: | | Receipt |
| Manufacture name: | Japan SLC | Sugidachi |
| Receive date: | | Receipt number: 034163 |
| Experimenter: | Atsuhiko Sugidachi | |
| Housing term: | | Number of rats: 36 |
| Receipt number: | 034163 | |

Additional 2 rats

↓

euthanasia using CO₂ gas

| | | |
|-----|---------|--|
| | B. W. | 72.0 mg |
| #1 | 271.0 g | |
| | 277.6 g | 11.3 mg |
| | 272.8 g | |
| | 275.0 g | |
| | 245.5 g | |
| #6 | 251.6 g | 1224.1 mg |
| #10 | 248.2 g | |
| #8 | 263.9 g | |
| #9 | 253.3 g | |
| #7 | 281.1 g | |
| #11 | 263.4 g | 5% Arabic gum = 50 mg/ml |
| #12 | 263.4 g | = 1224.1 / 24.48 ml dH ₂ O |
| #13 | 271.3 g | |
| | 265.3 g | |
| | 266.0 g | Aspirin |
| | 255.5 g | 10 mg/ml = 72.0 / 7.2 ml 5% Arabic gum soln. |
| | 245.6 g | |
| #18 | 272.4 g | |

CS 747

11.3 mg / 11.3 ml 5% Arabic gum soln. = 1 mg/ml
 1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 1ml = 0.6 mg/ml
 1 mg/ml soln. 1 ml + 5% Arabic gum soln. 2.33 ml = 0.3 mg/ml

heparin

1000 unit/ml soln. (origine) 1 ml + saline 9 ml = 100 unit/ml
 100 unit/ml soln. 3 ml + saline 7 ml = 30 unit/ml

| | B. W. (g) | Treatment | Measured value (mg) | Thrombus (mg) |
|----|-----------|--------------------|---------------------|---------------|
| #1 | 271 | V | 55.6 | 49.1 |
| 2 | 278 | A [10] | 52.9 | 46.4 |
| 3 | 273 | 747 [0.3] | 43.9 | 37.4 |
| 4 | 275 | 747 [0.6] | 41.0 | 34.5 |
| 5 | 246 | A [10] + 747 [0.3] | 24.6 | 18.1 |
| 6 | 252 | A [10] + 747 [0.6] | 19.7 | 13.2 |
| 7 | 281 | V | 59.0 | 52.5 |
| 8 | 264 | A [10] | 63.2 | 56.7 |
| 9 | 253 | 747 [0.3] | 47.1 | 40.6 |
| 10 | 248 | 747 [0.6] | 38.5 | 32.0 |
| 11 | 263 | A [10] + 747 [0.3] | 36.7 | 30.2 |
| 12 | 263 | A [10] + 747 [0.6] | 35.6 | 29.1 |
| 13 | 271 | V | 57.8 | 51.3 |
| 14 | 265 | A [10] | 44.3 | 37.8 |
| 15 | 266 | 747 [0.3] | 46.4 | 39.9 |
| 16 | 256 | 747 [0.6] | 42.2 | 35.7 |
| 17 | 246 | A [10] + 747 [0.3] | 48.3 | 41.8 |
| 18 | 272 | A [10] + 747 [0.6] | 36.6 | 30.1 |

V = Vehicle

A = Aspirin

747 = CS 747

#1 0.0556 g #7 0.0590 g #13 0.0578 g

#2 0.0529 g #8 0.0632 g #14 44.3 mg

#3 0.0439 g #9 0.0471 g #15 0.0464 g

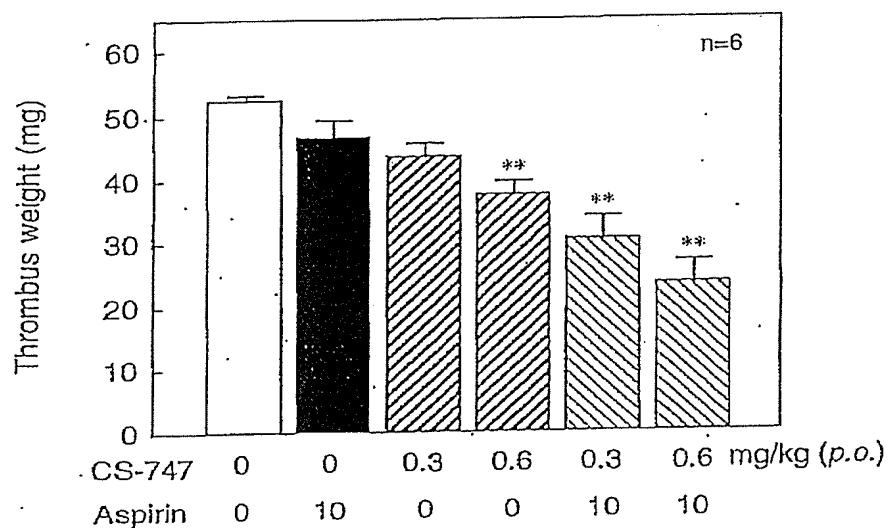
#4 0.0410 g #10 0.0385 g #16 42.2 mg

#5 0.0246 g #11 0.0367 g #17 48.3 mg

#6 0.0197 g #12 0.0356 g #18 36.6 mg

[REDACTED] A. Sugidachi [REDACTED] Hirose [REDACTED]

Arterio-venous shunt thrombosis model in rats



Aspirin + CS 747 Summary

| | |
|-----------------------------|------------|
| Vehicle | 52.3 ± 1.2 |
| Aspirin [10] | 46.6 ± 2.8 |
| CS 747 [0.3] | 43.5 ± 2.1 |
| CS 747 [0.6] | 37.5 ± 2.1 |
| Aspirin [10] + CS 747 [0.3] | 30.5 ± 3.5 |
| Aspirin [10] + CS 747 [0.6] | 23.2 ± 3.8 |

A. Sugidachi